REMARKS/ARGUMENTS

Claims 1-3, 9-12, 17-19, 38-40, 43-46, 49-52, and 55-64 are pending in the application. Reexamination and reconsideration of the claims in view of the following comments are respectfully requested.

THE REJECTION OF CLAIMS UNDER 35 U.S.C. §112, FIRST PARAGRAPH FOR LACK OF ENABLEMENT, SHOULD BE WITHDRAWN

Claims 1-3, 9-12, 17-19, 38, 43, 46, 49, 52, and 55-64 were rejected under 35 U.S.C. §112, first paragraph for lack of enablement. This rejection is respectfully traversed. For the reasons set forth below, the rejection of the claims under 35 U.S.C. §112 for lack of enablement should be withdrawn.

The Specification Fully Supports the Claims

The claimed sequences comprise SEQ ID NO:1 and sequences that have at least 90%, 93%, 94%, and 95% identity to SEQ ID NO:1. In addition, the claims require that the nucleotide sequences encode a polypeptide that is pesticidal for at least one pest belonging to the order Coleoptera. The specification teaches those skilled in the art how to make the claimed nucleotide sequences and provides examples of such sequences; (see, for example, pp. 11, 12, 13, 14, 18, 19, 25, and 65); guidance regarding alterations that allow the amino acid sequence to retain pesticidal activity (see, for example, p. 18 and pp. 19-20); methods for assaying the pesticidal activity of proteins (pp. 8 and 29, Example 4, Example 6, and Example 7); a discussion of Cry-8-like δ-endotoxins (SEQ ID NO:4 is a Cry-8-like δ-endotoxin) (pp. 24-25); guidance for determining percent identity of sequences (pp. 33-38); and, specific mutations that retain activity (Example 4 and Example 6).

The specification provides multiple truncated variants of SEQ ID NO: 1 and demonstrates that these truncated variants retrain activity. These sequences and the various methods for making and testing activity are discussed in the specification. However, for convenience, a summary of the sequences presented in the application are provided below in

Table 1. Briefly, SEQ ID NO:1 encodes the amino acid sequence set forth in SEQ ID NO:2. Active variants of SEQ ID NO:1 are set forth in SEQ ID NOS: 3, 15, 19, 11, 23, 31, 33, 29, 9, 43, 21, 39, 41, and, 45. SEQ ID NO:3 comprises a full length active variant of SEQ ID NO:1 and shares 92% sequence identity across the full length of SEQ ID NO:1. The remaining active variants are truncates of SEQ ID NO:1. For each truncated active variant, Table 1 provides both the global percent identity of the variant polynucleotide across the full length of SEQ ID NO:1 and a local percent identity across only the region of the truncate which shares homology to SEQ ID NO:1.

The specification provides fourteen (14) active variants of SEQ ID NO:1 which share between 38% and 92% identity across the full length of SEQ ID NO:1. When local alignments are performed between the truncated active variants and nucleotides 1 to 2007 of SEQ ID NO:1, the percent identity of the active variants to SEQ ID NO:1 ranges between 100% to 68% sequence identity. As multiple active variants have been provided which have a relationship to SEQ ID NO:1 well below the percent identities recited in the instant claims, the claims of the present invention are enabled.

While each of the 14 active variants of SEQ ID NO:1 provided in the specification provides clear support for enablement, a brief discussion of the active variants of SEQ ID NO:9 and 19 are provided to further emphasize the extent to which variants have been enabled.

SEQ ID NO:1 comprises 3621 nucleotides. SEQ ID NO:19 comprises 1860 nucleotides of SEQ ID NO:1 and continues to retain activity. The demonstration that such a fragment retains activity clearly illustrates that there is an increased likelihood that an alteration to one or more of the 1,761 additional nucleotides in SEQ ID NO:1 could be altered without disrupting function. Moreover, the specification provides further evidence that even the 1860 nucleotides which are set forth in SEQ ID NO:19 can be altered and still continue to retain activity. The active variant set forth in SEQ ID NO: 9 is the same truncate as SEQ ID NO:19 except that SEQ ID NO:9 contains maize optimized codons. As shown in the table below, SEQ ID NO:9 shares 38% global sequence identity to SEQ ID NO:1 and shares 68% local sequence identity and continues to retain activity. Accordingly, the data in the specification provides clear guidance to one of

skill in the art that active variants having at least 90%, at least 93%, at least 94%, and at least 95% sequence percent identity to SEQ ID NO:1 can be readily made.

The present Table does not have the "Description" column as was present in the Reply Brief as Applicants' shorthand notations for the sequences were confusing to the Examiner. However, all of the sequences presented below are set forth in the present specification. See, in particular, pages 14-16 for a description of the sequences.

The alignments summarized in Table 1 were performed using the Emboss Pairwise Alignment Algorithms using the Needle (global) alignment. This software is available from the European Bioinformatics Institute (EMBL-EBI) at http://www.ebi.ac.uk/emboss/align/#. Parameters employed in the protein alignments included a gap open of 10, an extended gap of 0.5 and the Blosum 62 matrix. Parameters employed in the nucleic acid alignments included a gap open of 10, an extended gap of 0.5 and the DNAfull matrix.

Table 1

SEQ ID NO for nucleotide sequence	Activity	Overall (global) % identity to SEQ ID NO:1	local % identity to nt 1-2007 of SEQ ID NO:1	Support in specification for activity	Corresponding polypeptide SEQ ID NO	Overall (global) % identity to SEQ ID NO:2	local % identity to aa 1-699 of SEQ ID NO:2
15	Activity against Colorado Potato Beetle	55%	100%	Table 1, page 68	16	56%	100%
19	Activity against Colorado Potato Beetle	52%	92.3%	Table 1, pp. 68-69	20	52%	92%
11	Activity against Colorado Potato Beetle, Southern Corn Rootworm	56%	99.4%	Table 1, pp. 68-69; Tables 2-4, pp. 70-71	12	56%	99.4%
23	Activity against Southern Corn Rootworm	56%	99.6%	Tables 2-4, pp. 70-71	24	56%	99.6%
31	Activity against Southern Corn Rootworm	51%	91.4%	Tables 2-4, pp. 70-71	32	51%	91.5%
33	Activity against Southern Corn Rootworm	51%	91.6%	Tables 2-4, pp. 70-71	34	60%	91.6%
29	Activity against Southern Corn Rootworm	51%	91.4%	Tables 2-4, pp. 70-71	30	51%	91.5%
9	Activity against Colorado Potato Beetle	38%	68.1%	Table 1, page 68	10	56%	100%

Table 1 (continued)

SEQ ID NO for nucleotide sequence	Activity	Overall (global) % identity to SEQ ID NO:1	local % identity to nt 1-2007 of SEQ ID NO:1	Support in specification for activity	Corresponding polypeptide SEQ ID NO	Overall (global) % identity to SEQ ID NO:2	local % identity to aa 1-699 of SEQ ID NO:2
43	Activity against Coleopteran Pests, data not shown	56%	99.6%	Page 27 of specification, lines 22-25	44	56%	99.6%
21	Activity against Coleopteran Pests, data not shown	56%	99.4%	Page 27 of specification, lines 22-25	22	56%	99.4%
39	Activity against Coleopteran Pests, data not shown	56%	99.4%	Page 27 of specification, lines 22-25	40	56%	99.4%
41	Activity against Coleopteran Pests, data not shown	51%	91.3%	Page 27 of specification, lines 22-25	42	51%	91.5%
45	Activity against Coleopteran Pests, data not shown	51%	91.6%	Page 27 of specification, lines 22-25	46	51%	91.6%
3	Activity against Coleopteran Pests, data not shown	92%	NA	Page 11, lines 15-19. examples 3 and 4	4	89%	NA

Accordingly, the specification provides exemplary sequences that fall within the scope of the claims as well as adequate guidance regarding making, testing, and identifying sequences that fall within the scope of the claims.

The Examiner Acknowledges the Broad Teachings of the Specification

The Office Action concludes that the specification "while being enabling for nucleic acids encoding SEQ ID NO:4, . . . does not reasonably provide enablement for any nucleic acid that has 90% identity to SEQ ID NO: 1...." Interestingly, the Examiner acknowledges that the specification does provide "guidance for methods of assaying the activity of *B. thuringiensis* strain 1218 and lysate against Western corn rootworm and Southern corn rootworm (examples 1 and 2); isolation of crystal protein from the strain and assaying of it for pesticidal activity against western corn root worm (example 3); identification of two coding regions, Cry1218-1 and Cry1218-2 (SEQ ID NO:1 and 3, with SEQ ID NOs:27 and 28 as the genomic clones). . . " Page 3, lines 4-8, of the Office Action. The Examiner further acknowledges that the specification teaches the "production of truncated proteins, SEQ ID NOs:16 and 18, encoded by SEQ ID NOs:16 and 18, encoded by SEQ ID NOs:15 and 17 respectively, in *E. coli* that are active against southern corn rootworm (example 4); and production of maize-preferred coding sequences of a different truncated version of Cry1218-1 – the nucleic acid is SEQ ID NO:9, which encodes SEQ ID NO:10 (example 5)." Page 3, lines 9-13, of the Office Action. The Examiner goes on to acknowledge that the specification "teaches making mutant versions of truncated Cry1218-1... all of which are effective against Colorado potato beetle (example 6)" or against southern and western corn rootworm (example 7). Page 3, lines 13-19, of the Office Action.

In view of these acknowledgments, Applicants respectfully submit that the present claims are enabled by the specification.

The Examiner's Reasoning is not Well Founded

In contrast to the conclusions stated in the Office Action (*e.g.*, page 4, first full paragraph), guidance is provided as to what sequence alterations may be made and still provide a

polypeptide species encompassed by the claim. Applicants have provided the exemplary nucleotide sequence of SEQ ID NO: 1 and the exemplary amino acid sequence of SEQ ID NO: 2. The claimed sequences of the invention vary from this sequence by structural parameters (*i.e.*, percent sequence identity to SEQ ID NO: 1). Guidance for determining percent identity of sequences is provided in the specification on pages 33 through 38.

Moreover, independent claims 1, 9, and 17, in addition to requiring a structural component (at least 90% sequence identity to the nucleotide sequence set forth in SEQ ID NO: 1), specify that the nucleotide sequence encodes a polypeptide which is pesticidal for at least one pest belonging to the order Coleoptera and therefore these claims (and claims dependent thereon) encompass functional variants. Guidance regarding alterations that allow the sequence to retain the specified pesticidal activity is also provided. See, for example, page 18, line 17 through page 19, line 20 that discuss variants and their pesticidal activity. In addition, methods for assaying pesticidal activity of proteins are routine in the art and are also described in the specification, for example on page 8, lines 24-30 and on page 28, lines 24-30 and in the experimental section in working examples such as Example 4, (pp. 65-66), Example 6 (pp. 67-69), and Example 7 (pp. 69-73).

The Examiner is referred to Table 1 provided herein. As explained above, the Table provides numerous examples of sequences, all of which are provided in the specification, some having less than the required sequence identity and having the required activity. Thus, the claims are enabled.

On page 6 of the Office Action, lines 3-20, the Examiner reasons that to enable the claims, one must make and test all possible combinations of nucleic acids falling within the scope of the claim.

> Making all possible single amino acid substitutions, in an 3621 nucleotide long nucleic acid like that of SEQ ID NO:1 would require making and analyzing 19^{3621} nucleic acids; these nucleic acids would have about 99.99% identity to SEQ ID NO:1. Because nucleic acids that have 90% identity to SEQ ID NO:1 would have up to 362 nucleotide substitutions, many more than 19^{3621} nucleic acids would need to be made and analyzed.

The Examiner's analysis is improper. As held by the court in *In re Borkowski*, 422 F.2d 904, 909, 164 U.S.P.Q. (BNA) 642, 645 (C.C.P.A. 1970), it is inappropriate "to study appellants' disclosure, to formulate a conclusion as to what he (the Examiner) regards as the broadest invention supported by the disclosure, and then to determine whether appellant's claims are broader than the Examiner's conception of what 'the invention' is." In the present case, the methods and examples disclosed in the specification readily teach one of skill in the art to make and test sequences having at least 90% identity to SEQ ID NO:1.

The specification provides guidance to one of skill in the art for making modifications, describes the domains of the Cry protein, and provides insights as to where modifications may be tolerated. See, for example, pages 23 and 24. The specification further teaches preparing modified sequences and testing such sequences for activity. *See*, for example, pages 23, 24, and 29, as well as, Examples 1, 6, and 7. Modified versions and truncated versions of the polypeptide are disclosed retaining pesticidal activity. See, for example, Table 1 provided herein.

The Examiner also ignores the information available in the art as of the filing date regarding δ-endotoxins. δ-endotoxins are extremely well-characterized and related to various degrees by similarities in their amino acid sequences and tertiary structures. The specification contains a reference to Li *et al.* as well as a discussion on designing mutant sequences. *See*, for example, page 25 of the specification:

> The inventors of the present invention used the solved structure of the *Cry3A* gene (Li *et al.* (1991) *Nature* 353:815-821) to produce a homology model of the Cry8 δ-endotoxin disclosed and claimed herein as SEQ ID NO:2 to gain insight into the relationship between structure and function of the endotoxin, and to design the recombinantly engineered proteins disclosed and claimed herein. A combined consideration of the published structural analyses of *B. thuringiensis* endotoxins and the reported function associated with particular structures, motifs, and the like indicates that specific regions of the endotoxin are correlated with particular functions and discrete steps of the mode of action of the protein. For example, δ-endotoxins isolated from *B. thuringiensis* are generally described as comprising three domains, a seven-helix bundle that is involved in pore formation, a three-sheet domain that has been implicated in receptor binding, and a beta-sandwich motif (Li *et al.* (1991) *Nature*, 305:815-821).

> The inventors reasoned that the toxicity of Cry8-like proteins, and specifically the toxicity of the Cry8 protein, could be improved by targeting the region located between alpha helices 3 and 4 of domain 1 of the endotoxin protein. This theory was premised both on the knowledge that alpha helices 4 and 5 of domain 1 of Cry3A δ-endotoxins had been reported to insert into the lipid bilayer of cells lining the midgut of susceptible insects (Gazit *et al.*, (1998) *PNAS USA* 95:12289-12294); the inventors' knowledge of the location of trypsin and chymotrypsin cleavage sites within the amino acid sequence of the wild-type protein; and the observation reported herein that the protein encoded by 1218-1 (i.e., SEQ ID NO:2) was more active against certain Coleopterans following *in vitro* activation by trypsin or chymotrypsin treatment. Accordingly, the inventors engineered a mutant Cry8-like protein that would comprise at least one additional trypsin cleavage site in the region located between helices 3 and 4 of domain 1.

Specification, p. 25, lines 1-24.

Yet, even in view of the description provided in the specification, the many examples taught, and the knowledge available in the art, the Examiner ignores all the teachings. The specification provides truncated polypeptides containing additional modifications that retain activity. The truncated variants lack nucleotides of SEQ ID NO:1 and the encoded protein retains activity.

The Examiner discounts all the disclosure and teachings and provides only flawed reasoning for her conclusion. The two references cited by the Examiner support Applicants' position that the claims are enabled, as discussed in more detail below.

Under the facts of the present application, one skilled in the art would understand whether a particular protein has at least 90%, 93%, 94%, or 95% sequence identity with SEQ ID NO:1 as set forth in the claims. In addition, functional assays are disclosed in the specification that provide sufficient guidance for one skilled in the art to determine whether a particular polynucleotide is within the scope of the claims. Thus, the claims are fully enabled.

At pages 7 and 8 of the Office Action, the Examiner lists several objections.

First, the Examiner notes that Table 1 has numerous errors. As noted above, Applicants have deleted the "Description" column of the table to avoid any confusion. The more important information in the Table is the % identity that the sequences share with the claimed sequences. The Examiner has further noted that in a previous response, SEQ ID NO:19 was indicated as

hiving different percent global identity to SEQ ID NO:1. In the previous response, the percent identity was listed as 51% whereas 52% is indicated in Table 1. The difference is simply a reflection of rounding off of the numbers. However, regardless of whether it is 51% or 52%, the variant clearly supports enablement for claims having at least 90% identity to SEQ ID NO:1.

Second, the Examiner notes that some of the sequences are truncated and that one of skill in the art would only look at it as a truncated protein. The Table provides % identity of the truncated nucleotide sequences and proteins across the full length of SEQ ID NO:1 and SEQ ID NO:2 as well as across the corresponding nucleotides of SEQ ID NO:1 and amino acids of SEQ ID NO:2. Thus, the truncated sequences establish that these is a region that can be deleted and yet the protein retains activity. One of skill in the art would recognize that this region would likely tolerate modifications in the deleted region. Further, as noted, even within the corresponding region, changes are tolerated.

Third, the Examiner notes that the specification does not consider deletions the same as substitutions. First, the claim does not require a deletion or a substitution. The claims are drawn to sequences having at least 90%, 93%, 94% or 95% sequence identity with SEQ ID NO:1. Secondly, the Examiner does not consider SEQ ID NO:3 which is a full length variant of SEQ ID NO:1 and has 92% sequence identity to SEQ ID NO:1.

Fourth, the Examiner would require Applicants to make and test every possible substitution. This is improper and counter to Federal Circuit and PTO Board authority for the Examiner to require Applicants to demonstrate every substitution that could be made in the sequence. While the claims encompass more, many changes can be made in the nucleotide sequence that would not change the encoded amino acid sequence. The Examiner is directed to SEQ ID NO:9 which shares only 68% local sequence identity and continues to retain activity.

Fifth, the Examiner indicates that SEQ ID NO:3 cannot provide support because "the vast majority of substitutions are localized to such a small area, the protein it encodes, SEQ ID NO:4 has 89% identity to SEQ ID NO:2. Thus, SEQ ID NO:3 cannot provide guidance for making nucleic acids with 90% identity to SEQ ID NO:1 and encoding a protein with 362 amino acid substitutions relative to SEQ ID NO:2" Page 8, lines 14-17. First, SEQ ID NO:3 provides support for substitutions that can be made to the nucleic acid sequence and the resulting protein

retain activity. Secondly, again the Examiner is misguided in requiring that Applicants demonstrate all the possible substitutions to provide enablement.

The Examiner Continues to Mischaracterize the Lazar and Hill References

The Examiner argues that making conservative amino acid substitutions does not produce predictable results and cites Lazar *et al.*, *Molecular & Cellular Biology* 8:1247-1252 (1988) in support of her position. The Examiner indicates that the conservative substitution of glutamic acid for aspartic acid at position 47 reduced biological function of transforming growth factor alpha while nonconservative substitutions with alanine or asparagine had no effect. The Examiner fails to consider the entire teachings of the reference.

First, the Lazar reference is drawn to studying transforming growth factor α (TGF-α). TGF-α is a mammalian polypeptide of 50 amino acids. The polypeptide is in no way related to the Cry proteins of the present invention. The reference relating to TGF-α does not bear any relevance to the claimed Cry proteins.

Secondly, with respect to the modifications described by Lazar, two amino acids of TGF-α which were known to be conserved among the family of EGF-like polypeptides were modified. It would come as little surprise to one skilled in the art that the modification of such a conserved amino acid should lead to the loss of function described by the authors. One of the changes at position 47 described by the authors indicates that [Asn-47]- TGF-α retains biological activity. The authors note that interestingly, two of the EGF-like viral proteins, myxomal growth factor and Shope fibroma growth factor, have Asn instead of Asp in position 47. Thus, the reference supports Applicants' position that protein domains are important and that by aligning sequences, one of skill in the art can determine what sites would likely tolerate changes.

On page 11 of the Office Action, the Examiner misinterprets the teachings of the Lazar reference. The purpose of the Lazar study was NOT to test "the commonly accepted notion that making conservative substitutions would not affect activity and that nonconservative substitutions would affect activity, even inactivate the protein." As explicitly set forth in the abstract, page 1247, the purpose of the Lazar work was "[t]o study the relationship between the primary structure of transforming growth factor α (TGT- α) and some of its functional

properties." To do so, conservative and nonconservative amino acid substitutions were made at two amino acid positions that are highly conserved. The results are not relevant to substitutions outside of conserved regions.

The Examiner additionally cites Hill *et al.*, *Biochemical & Biophysical Research Communications* 244:573-577 (1998) as supporting the position that substitution of a residue with a conservative amino acid can drastically reduce enzyme activity. The Examiner cites Hill as teaching that when three histidines that are maintained in ADP-glucose pyrophosphorylase across several species are substituted with the 'nonconservative' amino acid glutamine, there is little effect on enzyme activity, while the substitution of one of those histidines with the 'conservative' amino acid arginine drastically reduced enzyme activity.

First, the Hill reference is drawn to studying ADP-glucose pyrophosphorylase. The polypeptide is in no way related to the Cry proteins of the present invention. The reference relating to ADP-glucose pyrophosphorylase does not bear any relevance to the claimed Cry proteins.

Secondly, with respect to the modifications described by Hill, the modified residues were conserved among bacterial and plant ADP-glucose pyrophosphorylases. As set forth in the first line of the abstract, "[t]wo **absolutely conserved** histidines and a third **highly conserved** histidine are noted in 11 bacterial and plant ADP-glucose pyrophosphorylases." (emphasis added) These **absolutely** and **highly conserved** histidines were mutagenized and characterized in the paper. It would come as little surprise to one skilled in the art that the modification of one of these conserved amino acids should lead to the loss of function described by the authors.

The Examiner states on page 9, lines 15-16, that "hill *et al.* [sic] teach that conserved blocks cannot be relied on in making amino acid substitutions." The Examiner is completely mischaracterizing the reference. Even Hill states, "[c]omparisons of these sequences highlight those strictly conserved residues whose functions are essential." Hill *et al.* Page 573, column 2. The paper further notes that while the three conserved histidines are not essential for catalytic activity, when a substitution is made, activity is slightly lower than wild type. Page 576.

The paper does not speak to changes outside of conserved regions as taught in the present application.

The Examiner's References on Cry Proteins Support Applicants' Arguments

The Examiner indicates that making amino acid substitutions in Cry proteins is unpredictable. Three references, de Maagd *et al.* (1999) *Appl. Environ. Microbiol.* 65:4369-4374; Tounsi *et al.* (2003) *J. Appl. Microbiol.* 95:23-28; and Angsuthanasombat *et al.* (2001) *J. Biochem. Mol. Biol.* 34:402-407; are cited as supporting the Examiner's assertions. As discussed below, the Examiner misinterprets the references. When taken as a whole, the references support Applicants' claims.

The deMaagd reference is drawn to the identification of Cry1C domain III amino acid residues involved in insect specificity. As the article explains, progress has been made both in determining the three-dimensional structure of the toxin molecule and in identifying the primary sequences involved in specificity and receptor binding, allowing the study of structure-function relationships (page 4369, column 1). Domain III was known to be involved in binding of toxins to putative receptors of brush border membranes of insects. The study was undertaken to identify amino acid residues in domain III involved in specificity for beet armyworm. As noted in the abstract, the results "identify groups of amino acids as well as some individual residues in Cry1C domain III, which are strongly involved in *S. exigua*-specific activity as well as sometimes involved in *M. sexta*-specific activity." The work aligned domain III from Cry1C, Cry1E, and Bs21 and showed that only blocks B through E of Cry1C or parts thereof, are essential for a high level of activity against *S. exigua*. The work described in the publication was an effort to determine which parts of domain III of Cry1C are involved in *S. exigua*-specific activity. Individual amino acids were identified by mutagenesis that are involved in the specificity. The paper proves that with the knowledge in the art on Cry endotoxins, specific amino acids involved in binding specificity can be identified. Likewise, with this same knowledge, mutations can be made that retain activity. This work supports Applicants' arguments that changes can be made and function preserved.

The Tounsi reference reports on the study of the expression of a new Cry1Ia-type gene. By comparing the sequence of the new gene with known Cry1Ia genes, amino acid differences were detected (Figure 2, page 26). The paper notes that these substitutions may be important for

studies on toxicity. Again, the paper supports the principle that using alignments and domain information substitutions can be made in Cry proteins and activity retained.

The Angsuthanasombat paper reports on the directed mutagenesis of a Cry11a toxin. The paper notes that α4 and α5 of the 130kDa Cry 4B toxin are essential determinants of toxicity. Using amino acid sequence alignment with Cry1Aa and Cry3A and the homology model of Cry4B, the predicted α4 and α5 were located. To investigate the possible role for toxicity of charged and polar amino acids in α4 of Cry11A, eight Cry11A mutants were generated. As set forth on pg 405, only the R136A mutation resulted in total loss of larvicidal activity.

It is not surprising that a mutation in a critical region known to be essential for toxicity would have an effect on toxicity. The seven mutations did not abolish activity, but did affect the level of activity.

Again, the Examiner has mischaracterized the cited references which, if taken as a whole, support Applicants' position.

The Lazar, Hill, deMaagd, Tounsi and Angsuthanasombat References Support Applicants' Position That it is Within the Skill of the Art to Make and Test Modifications

The Lazar reference published in 1988 and the Hill reference published in 1998, both demonstrate that one of skill in the art well before 2000, the priority date of the present application, could make substitutions in polypeptide sequences and test for activity. Nothing more is required in the present application.

The deMaagd, Tounsi, and Angsuthanasombat references all make substitutions in Cry proteins and then test for activity. This is all that is required to test sequences that fall within the scope of the claims.

The specification teaches that a comparison of the amino acid sequences of Cry toxins of different specificities reveals five highly conserved sequence blocks. Structurally, the δ-endotoxins comprise three distinct domains, which are, from the N- to C-termini: a cluster of seven alpha- helices implicated in pore formation, three anti-parallel beta sheets implicated in cell binding, and a beta sandwich. *See* page 23 of the specification. The specification further teaches that a truncated protein can be made that retains activity. The truncated polypeptide

would lead one of skill in the art to conclude that the deleted region would tolerate modifications. Furthermore, one of skill in the art would appreciate that changes would be more likely to be tolerated outside of conserved domains. Thus, there is teaching in the specification that would guide one of skill in the art in making modifications. The specification further teaches preparing modified sequences and testing such sequences for activity. *See*, for example, pages 23, 24, and 29 as well as Examples 1, 6, and 7 of the Specification, and Table 1, above.

As assays for determining whether the modified sequences would retain activity were disclosed, one of skill in the art as of the filing date of the present application would have been able to make such modifications and test them for pesticidal activity. Nothing more is required to fully enable the claims. Accordingly, one of skill in the art would be able to determine the functionality of polypeptides encompassed by the claimed invention without resorting to undue experimentation and therefore the enablement requirement is satisfied.

That Some Experimentation May be Necessary Does Not Indicate That the Claims are Not Enabled

It is recognized that in unpredictable art areas, the court has refused to find broad generic claims enabled where the corresponding specifications only demonstrate the enablement of one or very few embodiments and do not demonstrate with reasonable specificity how to make and use other potential embodiments across the full scope of the claim. *See, e.g., In re Goodman*, 11 F.3d 1046, 1050-52, 29 U.S.P.Q.2d (BNA) 2010, 2013-15 (Fed. Cir. 1993); *Amgen, Inc. v. Chugai Pharm. Co.*, 927 F.2d 1200, 1212-14, 18 U.S.P.Q.2d (BNA) 1016, 1026-28 (Fed. Cir. 1991); *In re Vaeck*, 947 F.2d at 496, 20 U.S.P.Q.2d (BNA) at 1445. The court has explained that enablement is lacking in those cases because the undescribed embodiment cannot be made based on the disclosure in the specification, without undue experimentation. However, the court has made clear that the question of undue experimentation is a matter of degree. The fact that some experimentation is necessary does not preclude enablement; what is required is that the amount of experimentation "must not be unduly extensive." *Atlas Powder Co. v. E.I. DuPont de Nemours & Co.*, 750 F.2d 1569, 1576, 224 U.S.P.Q. (BNA) 409, 413 (Fed. Cir. 1984). The Patent and Trademark Office Board of Appeal has indicated: "the test is not merely quantitative,

since a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed to enable the determination of how to practice a desired embodiment of the invention claimed." *Ex Parte Jackson*, 217 U.S.P.Q. (BNA) 804, 807 (1982).

In the present case, all the Examiner has established is that some experimentation would be required to make and use other embodiments of the claimed invention. What the Examiner has not done is perform the fact-finding needed in order to reach a proper conclusion of undue experimentation. The Examiner has not relied upon any evidence in support of this rejection which would establish that making and testing other sequences beyond those described in the present specification amounts to undue experimentation. In fact, the Examiner has ignored the guidance in the specification, the presence of working examples, and the teachings of the prior art. The references cited by the Examiner support the position that the procedures described in Examples 4, 6, and 7 for making and testing modified sequences are routine in the art. The Examiner makes the rejection based upon unsupported opinions.

The Specification Meets the Federal Circuit Standard for Enablement

The Federal Circuit has repeatedly stated that enablement is not precluded by the necessity for some experimentation, so long as the experimentation needed to practice the invention is not undue. *In re Wands* 8 USPQ2d 1400 (Fed Cir 1988). Furthermore, a considerable amount of experimentation is permissible, if it is merely routine, or if the specification provides a reasonable amount of guidance in which the experimentation should proceed. *Id.*

Applicants stress that when evaluating the quantity of experimentation required, the court looks to the amount of experimentation required to practice a single embodiment of the invention, rather than the amount required to practice every embodiment of the invention, as the Office Action implies. For example, in *Wands*, the claims at issue were drawn to immunoassay methods using any monoclonal antibody having a binding affinity for HbsAg of at least 10^{-9} M. The PTO had taken the position that the claim was not enabled because undue experimentation

would be needed to make the monoclonal antibodies required for the assay. The Federal Circuit reversed and held that the claims were enabled, as the amount of experimentation required to isolate monoclonal antibodies and screen for those having the correct affinity was not undue. *See Id.* Clearly, the Federal Circuit did not contemplate that every antibody useful in the methods of the claim must be identified. Rather, the court considered the amount of experimentation required to identify one or a few monoclonal antibodies having the required affinity. *See also, Johns Hopkins University v. Cellpro*, 931 F. Supp. 303, 324 (D. Del. 1996), *aff'd in part, vacated in part, and remanded*, 47 USPQ2d 1705 (Fed. Cir. 1998) (stating that "[t]he specification need only enable one mode of making the claimed invention.").

In the instant case, the quantity of experimentation required to practice independent claim 1 amounts to two steps: generating a nucleotide sequence having at least 90% sequence identity to SEQ ID NO: 1 and assaying the encoded polypeptide for pesticidal activity against at least one pest belonging to the order Coleoptera. Such assays, while routine in the art, have further been presented in the specification. Similarly, the amount of experimentation needed to practice the other claims is not undue. For example, claim 9 recites a transformed plant comprising a nucleotide construct comprising a nucleotide sequence that has at least 90% sequence identity to the nucleotide sequence set forth in SEQ ID NO: 1 and that encodes a polypeptide that is pesticidal for at least one pest belonging to the order Coleoptera. Thus, in addition to the steps required to practice claim 1, claim 9 requires the transformation of a plant. Plant transformation is routine in the art and is also readily achieved by those of skill in the art. Thus, a rational scheme for practicing the claimed invention is provided.

Based on the guidance regarding the exemplary nucleotide and polypeptide sequences of the invention and the methods for determining whether a particular polypeptide has pesticidal activity against at least one insect of the order Coleoptera, the skilled artisan could choose among possible sequence modifications to produce polypeptides within the parameters set forth in the claims and then test these sequence variants to determine if they retained pesticidal activity. Consequently, contrary to the conclusions stated in the Office Action, the quantity of experimentation necessary and the amount of guidance presented in the specification is sufficient

to enable the claims. In view of this discussion, Applicants respectfully request that the rejection of claims under 35 U.S.C. §112, first paragraph, be withdrawn.

Moreover, Applicants note that recent Board of Appeals decisions support Applicants' arguments that the present claims meet the enablement requirement. See *Ex parte Sun*, No. 2003-1993 (Bd. Pat. App. Int., Jan. 20, 2004) and *Ex parte Vogelstein*, No. 2002-0779 (Bd. Pat. App. Int., Dec. 30, 2002). While these decisions are specifically indicated to not be precedential, Applicants note that similar enablement rejections of similar claims were reversed by the Board in view of similar support.

In establishing nonenablement, the burden rests initially with the Examiner to substantiate the unpredictability of the art and that, given the unpredictability, the specification does not provide sufficient information to guide those of skill to make and use the claimed invention across the full scope of the claims. In the present case, a clear goal is disclosed. Furthermore, guidance is provided for making the claimed sequences, assays are provided to determine whether modified sequences would encode proteins that retain activity, examples are provided showing that modifications to the nucleotide sequence can be made and the encoded proteins retain pesticidal activity, and art is cited that provides information on the Cry proteins of the invention. Thus, whatever unpredictability surrounds the construction of other sequences, the need for undue experimentation is mitigated by the examples of how to make and use such claimed sequences.

The Examiner repeatedly argues that the specification does not teach which amino acid substitutions can be made to retain pesticidal activity and that while the claims require that the nucleic acid encode pesticidal proteins the specification does not teach how to make such nucleic acids. These statements do not take into account the numerous truncated and mutant sequences set forth in the specification. As the Federal Circuit has noted, "That some experimentation may be required is not fatal, the issue is whether the amount of experimentation required is 'undue.'" *In re Vaeck*, 947 F.2d at 495, 20 U.S.P.Q.2d (BNA) at 1444.

For all these reasons, the rejection of the claims under 35 U.S.C. §112, first paragraph as lacking enablement should be withdrawn.

THE REJECTION OF THE CLAIMS UNDER 35 U.S.C. §112, FIRST PARAGRAPH FOR LACK OF WRITTEN DESCRIPTION, SHOULD BE WITHDRAWN

Claims 1-3, 9-12, 17-19, 38, 43, 46, 49, 52, and 55-64 were rejected under 35 U.S.C. §112, first paragraph, as failing to comply with the written description requirement. This rejection is respectfully traversed. For the reasons set forth below, the rejection of the claims under 35 U.S.C. §112, first paragraph, as failing to comply with the written description requirement should be withdrawn.

The Claimed Sequences Are Adequately Described in the Specification

The written description inquiry focuses on whether the specification reasonably conveys to one skilled in the art whether the applicant invented the claimed subject matter. Thus, the relevant inquiries are: What is the applicant's claimed invention? What is now claimed? The claimed invention is directed to nucleotide sequences having specific structural and biological properties. The specification provides both the DNA and amino acid sequences of a representative embodiment of the claimed sequences. Indeed, the Examiner has acknowledged that these claims drawn to specific sequences would be allowable if rewritten as independent claims. The specification also discloses modified sequences that fall within the scope of the claims. Accordingly, the application provides the structural features that characterize nucleic acid sequences having 90%, 93%, 94%, or 95% identity to SEQ ID NO:1 and still retain pesticidal activity. The sequences that fall within the scope of the claims can readily be identified by the methods set forth in the specification.

Table 1 provided with this response provides a guide of sequences disclosed in the specification. As one can see from the Table, one of skill in the art would readily understand that Applicants were in possession of the claimed invention. The Table provides sequences that are modified yet still retain activity. Accordingly, the claims are fully described in the specification.

Applicants note that the description of a representative number of species does not require the description to be of such specificity that it would provide individual support for each species that the genus embraces. 66 Fed. Reg. 1099, 1106 (2001). Satisfactory disclosure of a "representative number" depends on whether one of skill in the art would recognize that the

applicant was in possession of the necessary common attributes or features of the elements possessed by the members of the genus in view of the species disclosed. 66 Fed. Reg. 1099, 1106 (2001). Applicants submit that the knowledge and level of skill in the art would allow a person of ordinary skill to envision the claimed invention, *i.e.*, a nucleotide sequence having at least 90% sequence identity to the sequence set forth in SEQ ID NO: 3.

The description of a claimed genus can be by structure, formula, chemical name, or physical properties. *See, Ex parte Maizel*, 27 USPQ2d 1662, 1669 (B.P.A.I. 1992), citing *Amgen v. Chugai*, 927 F.2d 1200, 1206 (Fed. Cir. 1991). A genus of DNAs may therefore be described by means of a recitation of a representative number of DNAs defined by nucleotide sequence and falling within the scope of the genus, *or* by means of a recitation of structural features common to the genus, which features constitute a substantial portion of the genus. *See, Regents of the University of California v. Eli Lilly & Co.*, 119 F.3d 1559, 1569 (Fed. Cir. 1997) (referred to herein as "*Lilly*"); *see also* Guidelines for Examination of Patent Applications Under the 35 U.S.C. 112, First Paragraph, "Written Description" Requirement, 66 Fed. Reg. 1099, 1106 (2001) (referred to herein as the "Guidelines"). The Office Action cites *Lilly* but ignores this aspect of the case as well as the Guidelines. Indeed, the passages cited in the Office Action refer to the description of a sequence by function alone without any structural limitation, which is not the case here. All of the pending claims recite a functional limitation and also require a predictable structure of at least 90% sequence identity to SEQ ID NO: 3. Under both Lilly and the Guidelines, these requirements for function in combination with the recitation of a predictable structure should be sufficient to satisfy the written description requirement.

Applicants note that the Federal Circuit has explicitly stated that

> *Eli Lilly* did not hold that all functional descriptions of genetic material necessarily fail as a matter of law to meet the written description requirement; rather, the requirement may be satisfied if in the knowledge of the art the disclosed function is sufficiently correlated to a particular, known structure.

Amgen, Inc. v. Hoechst Marion Roussel, Inc., 314 F.3d 1313, 1332 (Fed. Cir. 2003). *See also, Moba, B.V. v. Diamond Automation, Inc.*, 325 F.3d 1306, 1320 (noting that "[i]n more recent cases, however, this court has distinguished *Lilly*" and further noting that in *Enzo Biochem, Inc.*

v. Gen-Probe, Inc., 323 F.3d 956 (Fed. Cir. 2002), "neither the specification nor the deposited biological material recited the precise 'structure, formula, chemical name, or physical properties' required by *Lilly*.")

Example 14 of the "Synopsis of Application of Written Description Guidelines" is directed to a generic claim: a protein having at least 95% sequence identity to the sequence of SEQ ID NO: 3, wherein the sequence catalyzes the reaction A→B. The synopsis materials conclude that the generic claim of Example 14 is sufficiently described under §112, first paragraph, because: 1) "the single sequence disclosed in SEQ ID NO: 3 is representative of the genus"; and 2) the claim recites a limitation requiring the compound to catalyze the reaction from A→B. The synopsis materials conclude that one of skill in art would recognize that the Applicants were in possession of the necessary common attributes possessed by the members of the genus.

Following the analysis of Example 14, Applicants submit that the present claims satisfy the written description requirements of § 112, first paragraph. Specifically, the claims of the present invention encompass sequences having at least 90% sequence identity to SEQ ID NO: 3, wherein the encoded polypeptide is pesticidal for at least one pest belonging to the order Coleoptera. As in Example 14, the specification discloses the nucleic acid sequence of SEQ ID NO: 3 and claims recite a limitation requiring the compound to have a specific function (*i.e.*, pesticidal activity). Consequently, contrary to the conclusion stated in the Office Action, the sequences encompassed by the claims are defined by relevant identifying physical and chemical properties. In fact, the common attributes or features of the elements possessed by the members of the genus is that they encode polypeptides having pesticidal activity against at least one pest of the order Coleoptera and share at least 90% sequence identity at the nucleotide level to the disclosed nucleotide sequence of SEQ ID NO: 3. The necessary common features of the claimed genus are clear.

In summary, the description of a representative number of species *does not* require the description to be of such specificity that it would provide individual support for each species that the genus embraces. Applicants submit that the relevant identifying physical and chemical properties of the disclosed genus would be clearly recognized by one of skill in the art and

consequently, the Applicant was in possession of the necessary common attributes or features of the elements possessed by the members of the genus. Accordingly, the rejection of claims 1-2, 5-12, and 15-18 under 35 U.S.C. §112, first paragraph, for lack of written description should be withdrawn.

The Examiner Does not Consider the Written Description Support in the Specification

As set forth above, there is adequate written description support in the specification for the claims. The Examiner argues that "[t]he only species reduced to practice in the specification is SEQ ID NO:1, which encodes SEQ ID NO:2." The Examiner is overlooking SEQ ID NOs: 1, 15, 19, 11, 23, 31, 33, 29, 9, 43, 21, 39, 41, and 45. See Table 1, included above. The Table includes the overall % identity and the local % identity of the sequences with SEQ ID NO:1. As set forth in the Table, SEQ ID NOs: 1 and 3 represent sequences having 92% sequence identity. Additionally, the specification provides multiple truncated variants and demonstrates that these truncated variants retrain activity. These sequences demonstrate that sequences having at least 90% sequence identity to SEQ ID NO:1 can be made and retain activity. As Table 1 demonstrates, variant sequences not only can be made that retain activity but are disclosed within the specification. A summary of the sequences presented in the application are provided in Table 1. Briefly, SEQ ID NO:1 encodes the amino acid sequence set forth in SEQ ID NO:2. Active variants of SEQ ID NO:1 are set forth in SEQ ID NOS: 3, 15, 19, 11, 23, 31, 33, 29, 9, 43, 21, 39, 41, and, 45. SEQ ID NO:3 comprises a full length active variant of SEQ ID NO:1 and shares 93% sequence identity across the full length of SEQ ID NO:1. The remaining active variants are truncates of SEQ ID NO:1. For each truncated active variant, Table 1 provides both the global percent identity of the variant polynucleotide across the full length of SEQ ID NO:1 and a local percent identity across only the region of the truncate which shares homology to SEQ ID NO:1.

The specification provides fourteen (14) active variants which share between 38% and 92% identity across the full length of SEQ ID NO:1. When local alignments are performed between the truncated active variants and nucleotides 1 to 2007 of SEQ ID NO:1, the percent identity of the active variants to SEQ ID NO:1 ranges between 68% to 100% sequence identity.

As multiple active variants have been provided which have a relationship to SEQ ID NO:1 well below the percent identities recited in the instant claims, the written description requirement is met.

The Examiner's statement that "Applicants have [sic] not, in fact, described nucleic acids with 90%, 93%, 94% or 95% identity to SEQ ID NO:1 and that encode a protein pesticidal for at least one pest belonging to the order Coleoptera within the full scope of the claims, and the specification fails to provide an adequate written description of the claimed invention." is incorrect. Page 14, lines 16-19, of the Office Action. Table 1 and the 14 sequences contained therein, all of which are set forth in the specification, indicate otherwise. Accordingly, the claims are fully described in the specification.

The Facts of the Present Case Are Distinguishable from Lilly and Fiers

The Examiner quotes *Eli Lilly* at page 1406 stating "A [*sic*] description of a genus of cDNAs may be achieved by means of a recitation of a representative number of cDNAs, defined by nucleotide sequence. . ." First, as noted above, the specification provides more information than merely reciting SEQ ID NO:1. The specification provides: nucleotide sequences that fall within the scope of the claims (see, for example, pp. 11, 12, 13, 14, 18, 19, 25, and 65 and Table 1), guidance regarding alterations that allow the amino acid sequence to retain pesticidal activity (see, for example, p. 18 and pp.19-20); methods for assaying the pesticidal activity of proteins (pp. 8 and 29, Example 4, Example 6, and Example 7); a discussion of Cry-8-like δ-endotoxins (SEQ ID NO:2 is a Cry-8-like δ-endotoxin) (pp. 24-25); guidance for determining percent identity of sequences (pp. 33-38); and, specific mutations that fall within the scope of the claimed invention (Example 4 and Example 6).

Secondly, the Examiner's appeal to *Eli Lilly* is misplaced. As noted by the Federal Circuit in *Invitrogen Corp. v. Clontech Laboratories, Inc.* 77 U.S.P.Q.2d (BNA) 1161, 1175 (Fed. Cir. 2005), "[i]n those cases . . . , *University of California v. Eli Lilly & Co.*, 119 F.3d 1559, 43 U.S.P.Q.2d (BNA) 1398 (Fed. Cir. 1997) and *Fiers v. Revel*, 984 F.2d 1164, 1171, 25 U.S.P.Q.2d (BNA) 1601, 1606 (Fed. Cir. 1993), the patent specifications at issue did not identify the sequence (structure) of any embodiment of DNA claimed therein. . . . In contrast, the

shared written description for the patents-in-issue recites both the DNA and amino acid sequences of a representative embodiment of the claimed RT enzyme. The specification also discloses test data that the enzyme produced by the listed sequence has the claimed features--DNA polymerase activity without RNase H activity. Under both the *Eli Lilly* and *Fiers* analysis, the specification at bar is sufficient." *Id.* at 1073.

In the present application, a representative nucleic acid and amino acid sequence is provided. Additionally, modified sequences are disclosed which are representative of the claimed sequences. Accordingly, the application meets the requirement for written description for the claimed sequences.

In view of the above arguments and amendments, all grounds for rejection under 35 U.S.C. §112, first paragraph, have been overcome. Accordingly, it is respectfully submitted that the rejections under 35 U.S.C. §112, first paragraph, should be withdrawn.

CONCLUSION

In view of the above amendments and remarks, Applicants submit that the rejections of the claims under 35 U.S.C. §112, first paragraph are overcome. Applicants respectfully submit that this application is now in condition for allowance. Early notice to this effect is solicited.

If in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject Application, the Examiner is invited to call the undersigned.

It is not believed that extensions of time or fees for net addition of claims are required, beyond those that may otherwise be provided for in documents accompanying this paper. However, in the event that additional extensions of time are necessary to allow consideration of this paper, such extensions are hereby petitioned under 37 CFR § 1.136(a), and any fee required therefore (including fees for net addition of claims) is hereby authorized to be charged to Deposit Account No. 16-0605.

It is not believed that extensions of time or fees for net addition of claims are required, beyond those that may otherwise be provided for in documents accompanying this paper.

Appl. No.: 10/032,717
Amdt. dated May 18, 2006
Reply to Office Action of August 11, 2006

However, in the event that additional extensions of time are necessary to allow consideration of this paper, such extensions are hereby petitioned under 37 CFR § 1.136(a), and any fee required therefore (including fees for net addition of claims) is hereby authorized to be charged to Deposit Account No. 16-0605.

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LEGAL01/2207317v1